

FIG. 2A

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Protocol

Cycle 1: (1X)

Step 1: 95.0C for 01:00

Cycle 2: (40X)

Step 1: 95.0C for 00:20

Step 2: 53.0C for 00:20

Step 3: 68.0C for 00:20

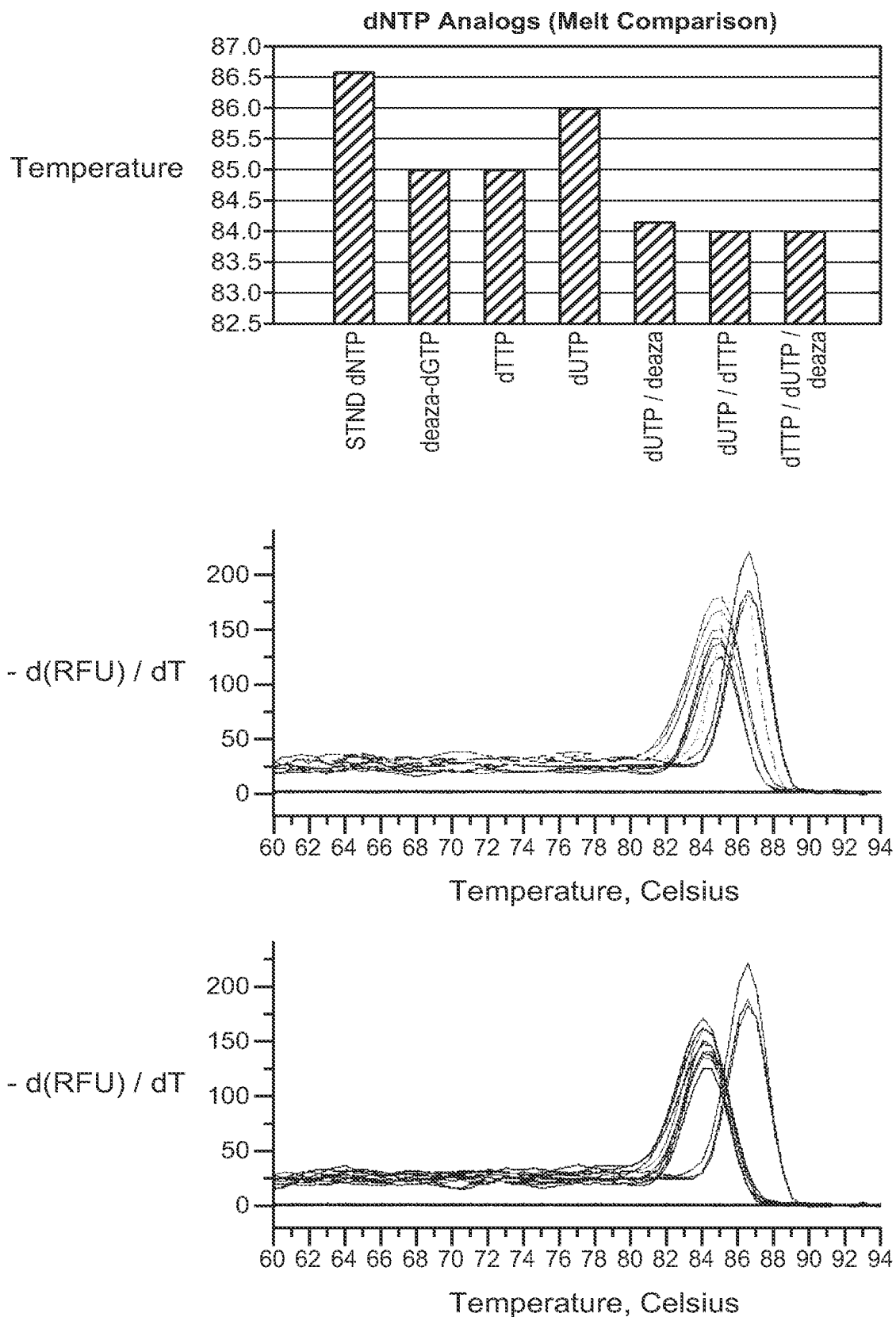
Cycle 3: (1X)

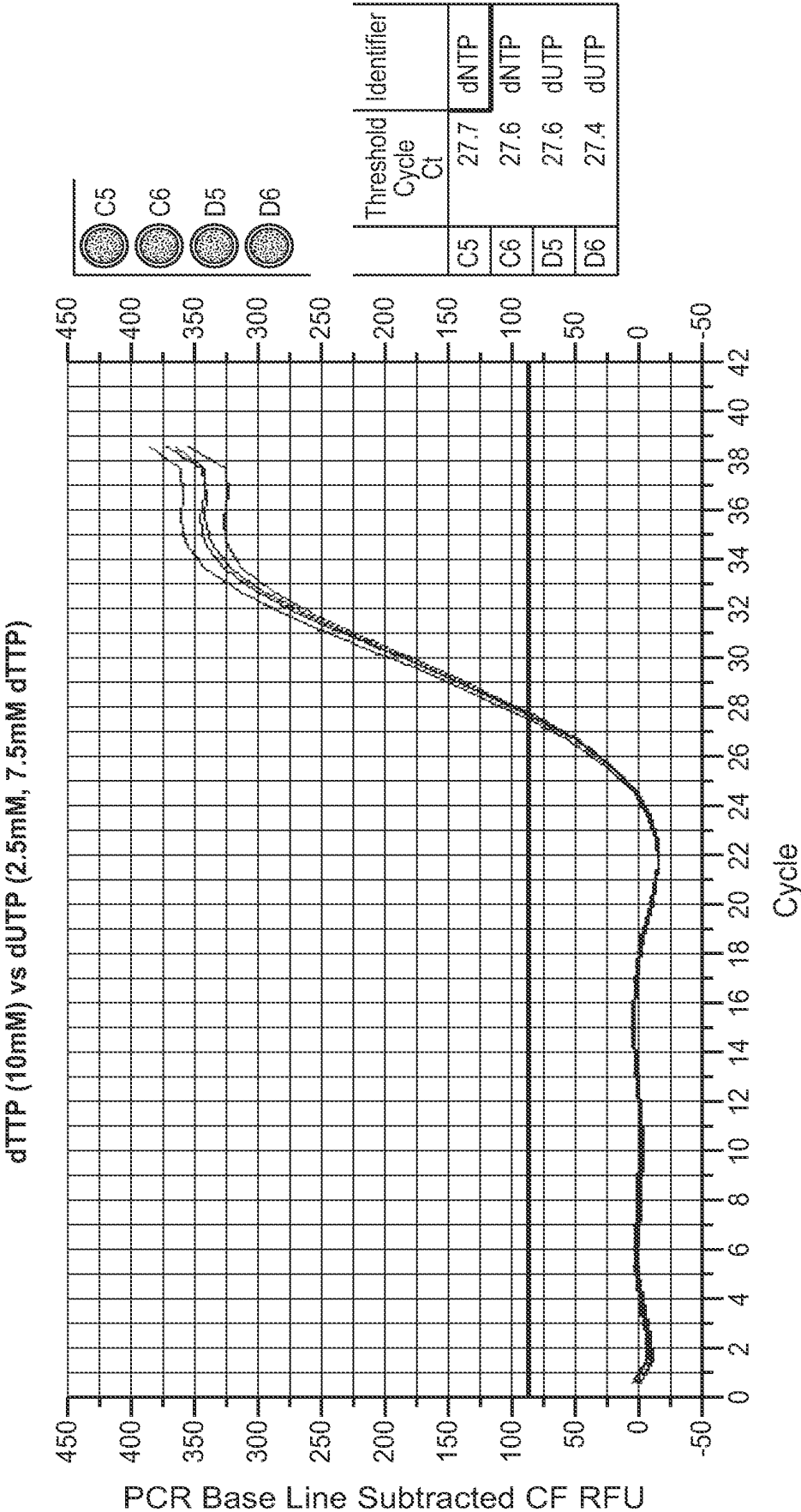
HotMaster Taq Reactions w/ STND dNTPs		
Reaction Component	Initial Concentration or Volume	Final Concentration/Volume
QuantMaster Probe Buffer	10X	1X
dNTP Mix		
dATP	10mM	200uM
dCTP	10mM	200uM
dGTP	10mM	200uM
dTTP	10mM	200uM
FactorVIII Forward Primer	10uM	200nM
FactorVIII Reverse Primer	10uM	200nM
HotMaster Taq Polymerase	5U/ul	1U
MBGW	N/A	36.8 - 38.8 uL
*Human gDNA (Promega)	25ng/uL	50ng
* Not Included in NTCs		

HotMaster Taq Reactions w/ 20% dUTP Mix		
Reaction Component	Initial Concentration or Volume	Final Concentration/Volume
QuantMaster Probe Buffer	10X	1X
dNTP Mix		
dATP	10mM	200uM
dCTP	10mM	200uM
dGTP	10mM	200uM
dTTP	8mM	160uM
dUTP	2mM	40uM
FactorVII Forward Primer	10uM	200nM
FactorVIII Reverse Primer	10uM	200nM
HotMaster Taq Polymerase	5U/ul	1U
MBGW	N/A	36.8 - 38.8 uL
*Human gDNA (Promega)	25ng/uL	50ng
* Not Included in NTCs		

FIG. 2B

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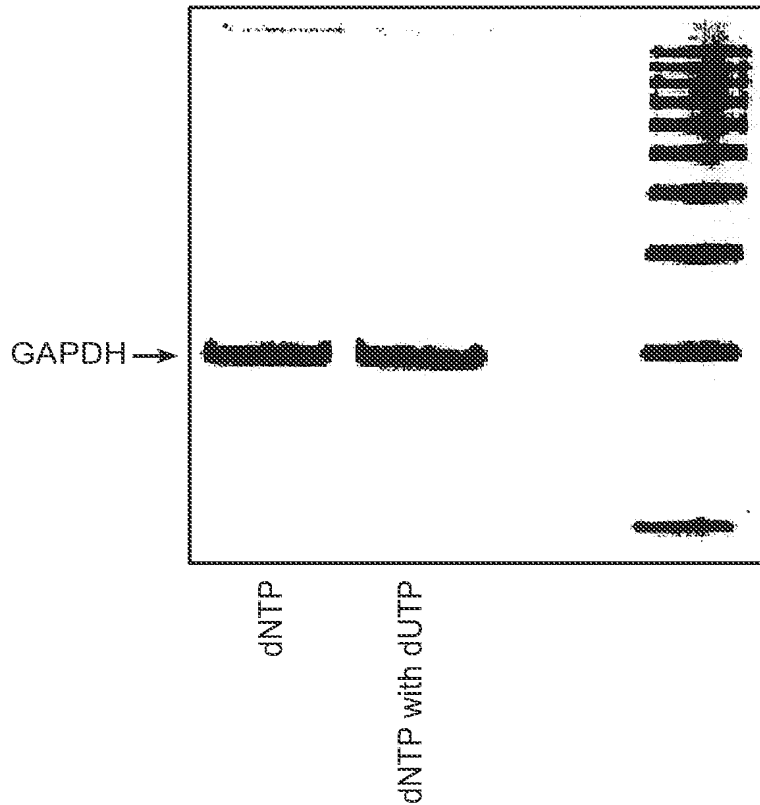
**FIG. 3**



In real-time RT-PCR, the use of dTTP or combination of dTTP and dUTP does not seem to affect the Ct or RFU significantly.

FIG. 4A

Standard dNTP Mix with dTTP (10mM) vs dUTP Mix
(2.5mM dUTP, 7.5mM dTTP)

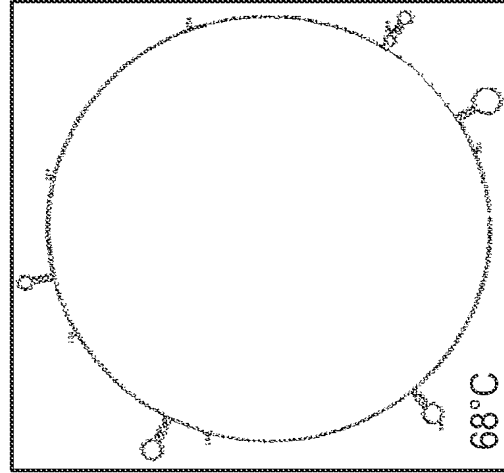
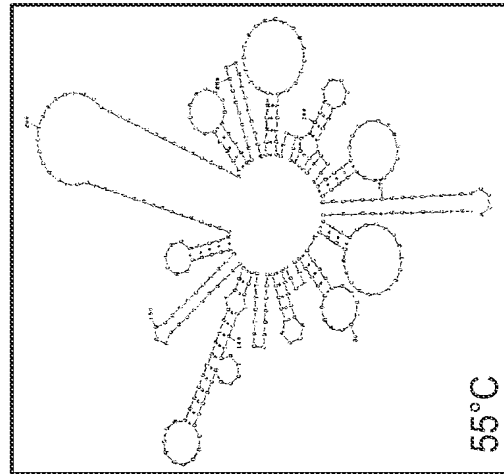
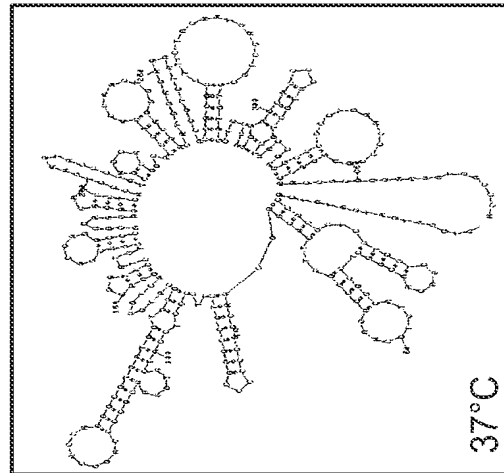


These results show that the addition of dUTP to the dNTP mix does not significantly affect the product yield.

FIG. 4B

Beta-Actin mRNA Sequence

```
atggatgatatcgccggcgctgctgacaaaggctccggcatgtgcaaggcgggttcgcgggcga  
cgatgccccggcgctcttccctccatcgtggggcgccccaggcaccaggcgtgatggtgggcattg  
ggtcagaaggattcctatgtgggcgacgagggccagagcaagagaggcatcctcacctgaagtaccca  
tcgagcacggcatcgtcaccaactgggacgacatggagaaaaatctggcacccacaccttctacaatgagctg  
cgtgtggctcccgaggacccccgtgctgctgacgaggccccctgaacccaaggggccaaccgcga  
gaagatgaccca
```

**FIG. 5A**

Beta-Actin Partial Gene Sequence w/ Intron

```

atggatgatatcgccgctcgtcgtcgacaacggctccggcatgtgcaaggccggcttcggggcga
cgatccccccggcgtcttccctccatcgtggggcccccaggcaccag|gtagggagctggctgg
gtgggcagccccgggagcggggaggcaaggcgctttctctgcacaggagcctcccgtttccgg
ggtgggtgcgccgtgctcagggtttctgtccttctccctccag|ggcgtgatggggcatgggtcagaa
ggattcctatgtggcgacgagggccagagagagagggcatcctcacctgaagtaccccatcgagcac
ggcatcgtcaccactgggacgacatggagaaaatctggcaccacacacctctacaatgagctcgtggct
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ccca

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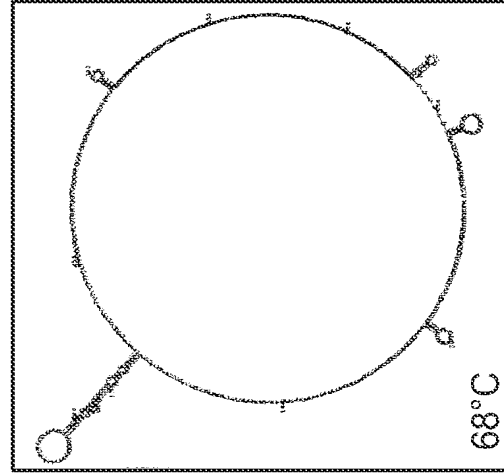
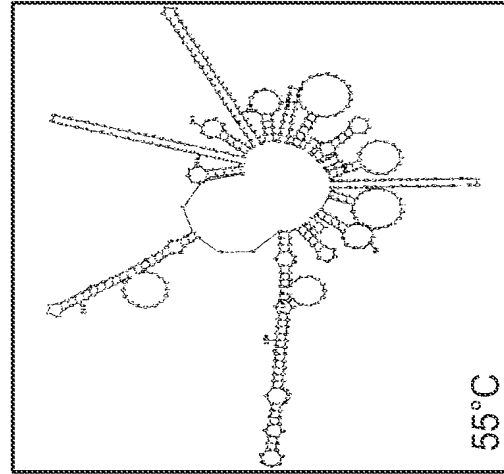
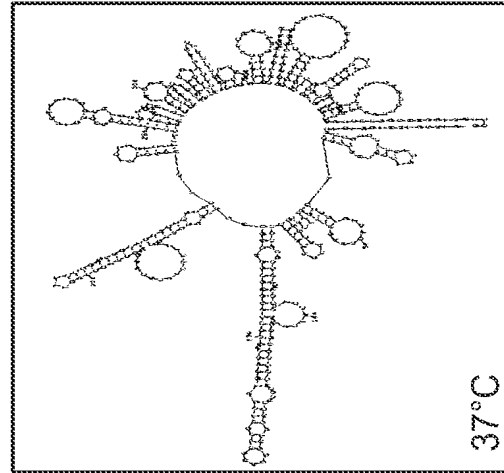
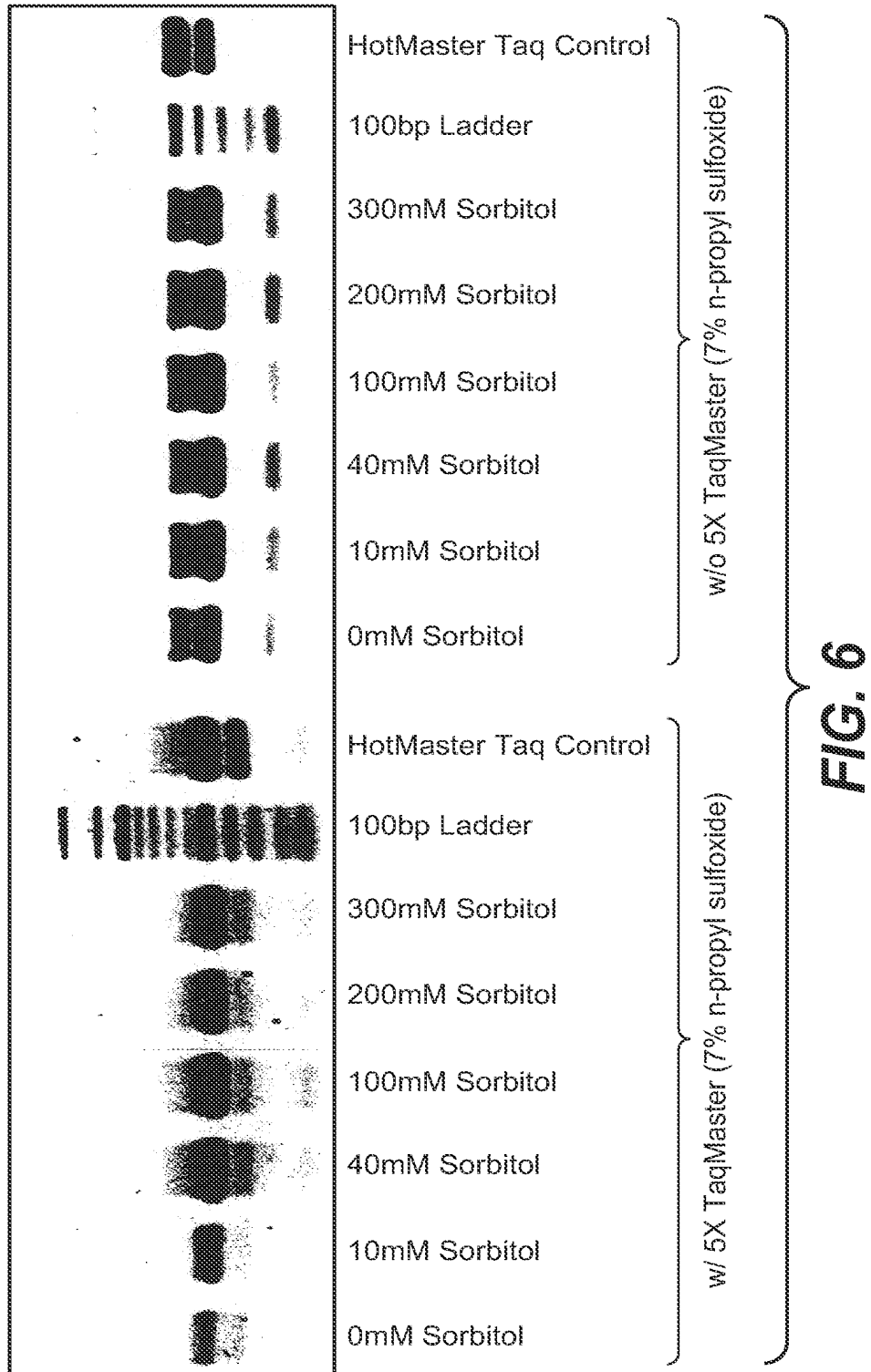


FIG. 5B



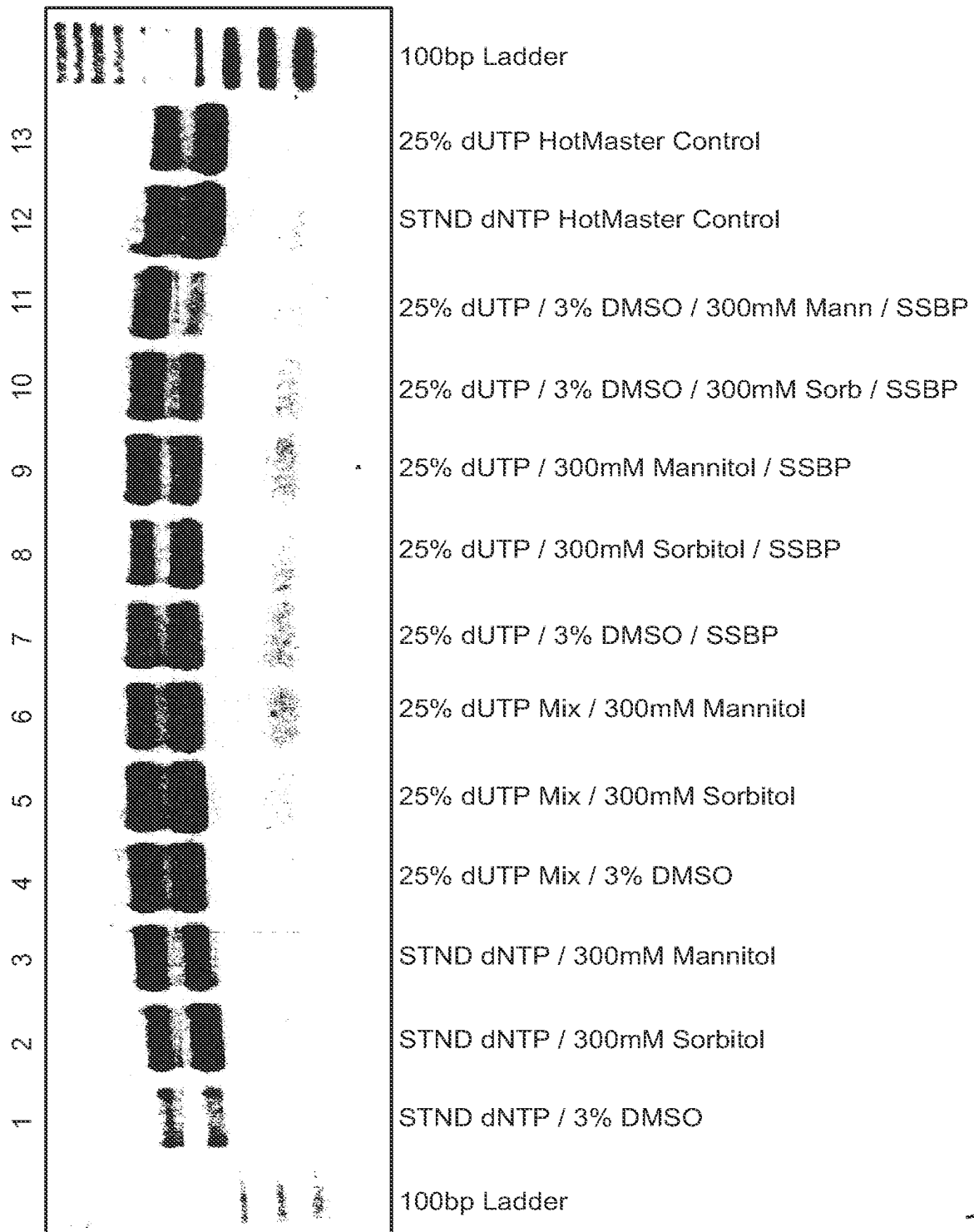
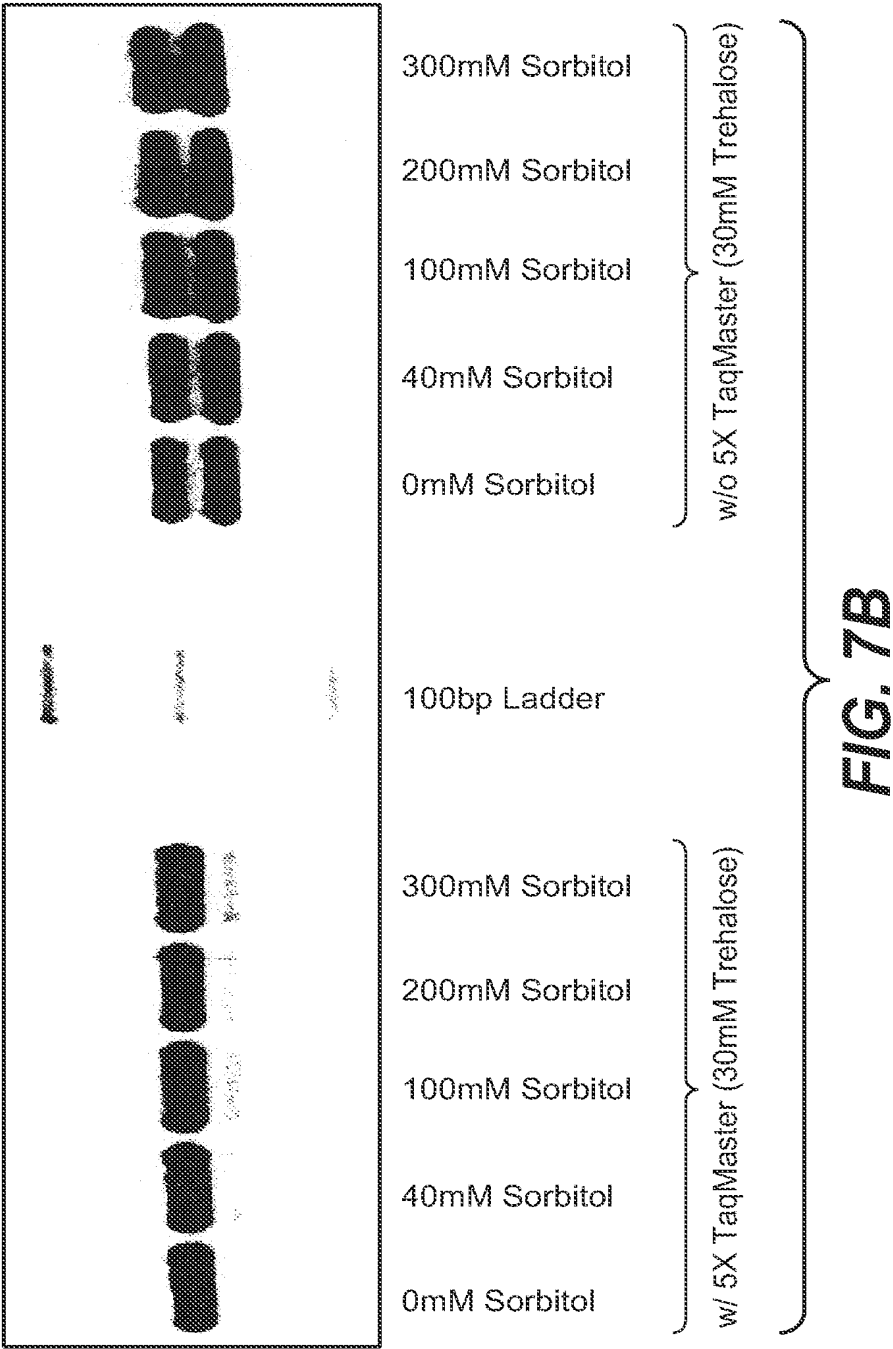


FIG. 7A



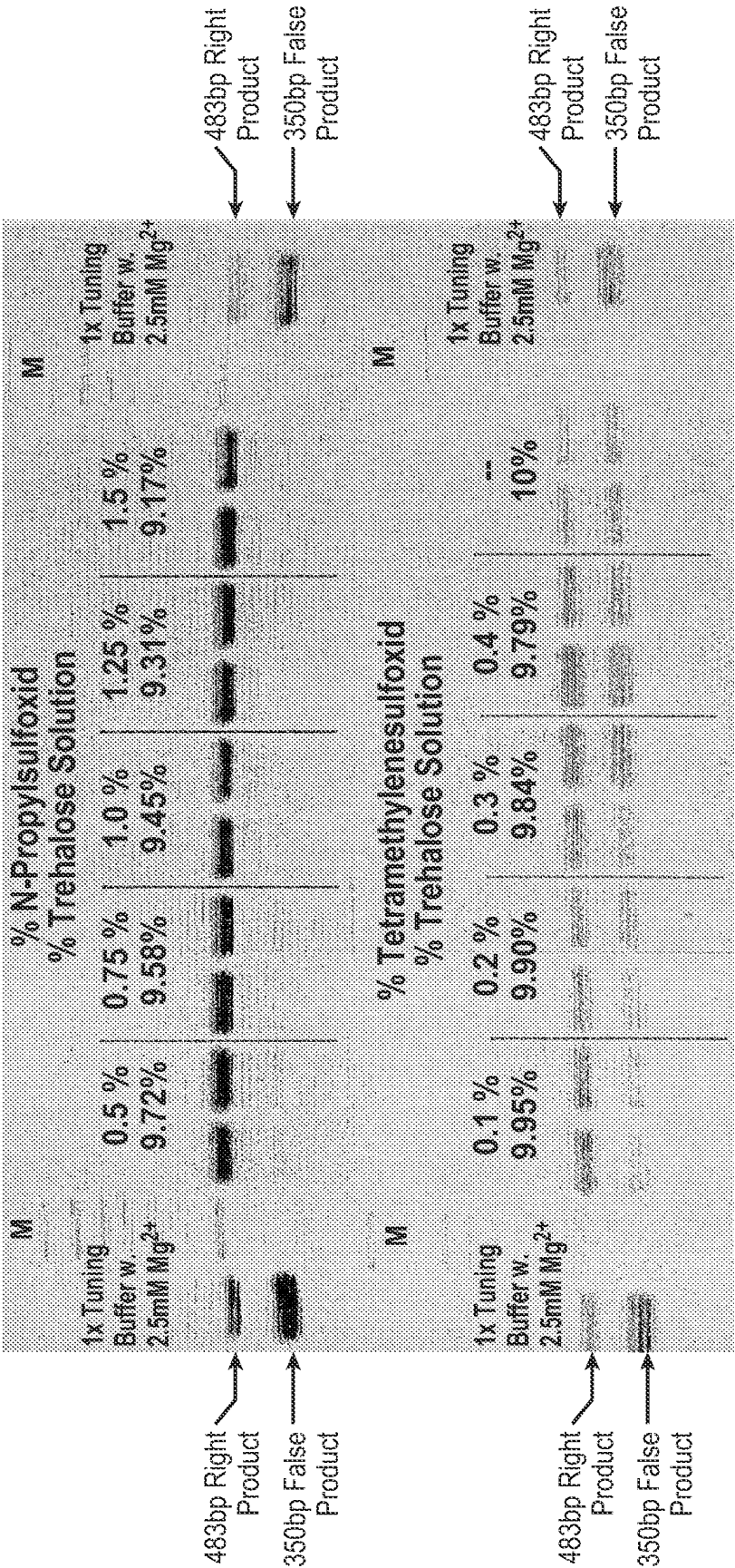


FIG. 8

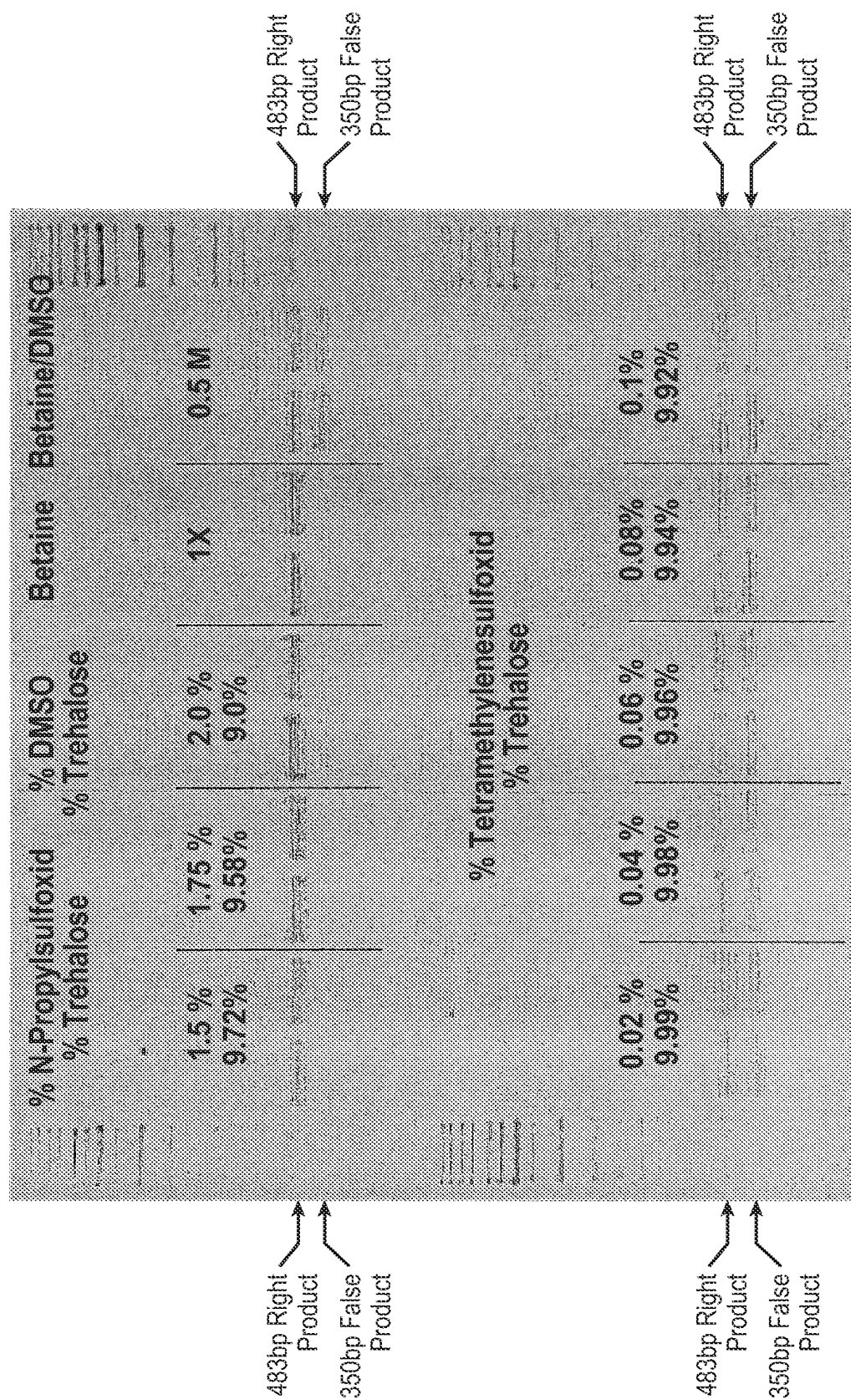


FIG. 9